Spiroscytalin, a New Tetramic Acid and Other Metabolites of Mixed Biogenesis from *Scytalidium cuboideum*

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Abstract:

Spiroscytalin (**1**), a new tetramic acid that possesses an uncommon spiro-ring fusion between a polyketide-derived octalin ring system and a 2,4-pyrrolidinedione, along with two known compounds, leporin B (**2**) and purpactin A (**3**), were isolated from a solid phase culture of the fungus *Scytalidium cuboideum* (MSX 68345). The molecular connectivity of **1**–**3** was determined using NMR spectroscopy and mass spectrometry. The relative configurations of **1** and **2** were determined by NOESY experiments. The absolute configuration of **1** was determined by electronic circular dichroism (ECD) via a combination of experimental measurements and computational calculations. While leporin B was known, it displayed activities that had not been reported previously, including cytotoxicity against three human tumour cell lines and antibacterial activity against *Candida albicans* and *Staphylococcus aureus*.

Keywords: Tetramic acid | Leporin B | Purpactin A | *Scytalidium cuboideum*

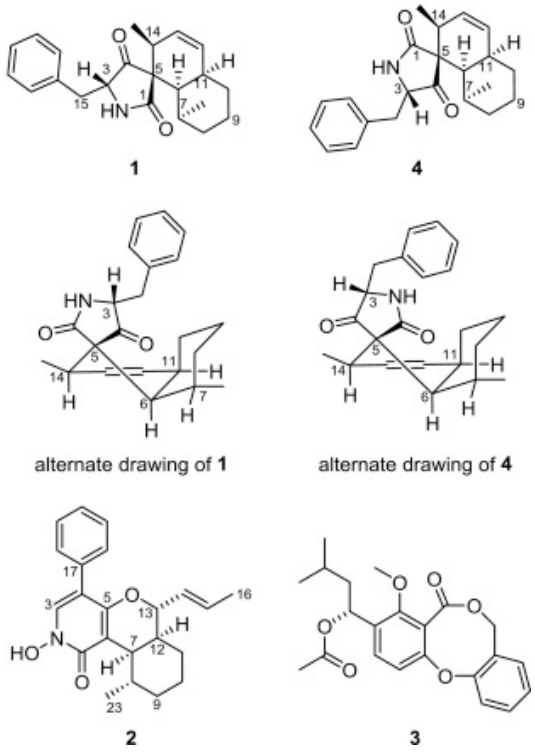
Article:

1. Introduction

Ongoing studies on filamentous fungi as a source of anticancer leads[1, 2] has provided our team with a view of the broad structural diversity that can be uncovered.[3] Natural products from mixed biogenetic origin[4, 5, 6, 7, 8] add to this chemical diversity, as they result from the merging of precursors from two distinct biosynthetic pools (i.e., acetylCoA and amino acids). Tetramic acids[6] represent a good example, due to their heterocyclic 2,4-pyrrolidinedione ring system, and

this compound class has displayed a variety of biological activities, ranging from HIV-1 integrase inhibitors[5] to antibacterial and antifungal activities[4]to cytotoxicity.[6] These activities, in turn, have spawned further interest in discovering new members of the class[9, 10] and paved the way for synthetic studies of natural products[11, 12, 13] and derivatives thereof.[14, 15, 16] A bioactivity-directed purification study on a solid phase culture of *Scytalidium cuboideum* (MSX 68345) afforded three bioactive compounds, two of which were of mixed biogenetic origin. The new compound, spiroscytalin (**1**), was from the tetramic acid class, while one of the known compounds, leporin B (**2**), was an *N*-hydroxy-2-pyridone alkaloid;[8, 17] the other known compound was purpactin A (**3**).

Structures



2. Results and discussion

Compound **1** was assigned the molecular formula $C_{22}H_{27}NO_2$ via HRESIMS data (*m*/*z*338.2113 for $[M+H]^+$), establishing an index of hydrogen deficiency (IHD) of 10. The ^{1}H NMR data (Table 1, Fig. S1) showed resonances for a monosubstituted aromatic ring (δ_H7.22–7.35 for H-17 to H-21) as well as a methylene unit (δ_H 3.35 and 2.51 for H_2-15) connected to it by virtue of HMBC correlations, suggestive of a phenylalanine moiety. Two olefinic protons were also observed (δ_H 5.77 and 5.34 for H-12 and H-13, respectively) as well as five methine protons (δ_H 3.78, 2.28, 1.90, 2.46, and 2.73 for H-3, H-6, H-7, H-11, and H-14, respectively), six more methylene protons (δ_H 1.38, 1.27; 1.49×2; and 1.78, 1.55 for H_2-8, H_2-9, and H_2-10, respectively), and two methyl groups that were each doublets (δ_H 1.13 and 0.89, for CH_3-7 and CH_3-14, respectively). Finally, one NH proton was observed at δ_H 6.41. The ^{13}C NMR data (Table 1) showed resonances for 22 carbons, supportive of the molecular formula, including signals for a ketone carbonyl (δ_C 212.2 for C-4), an amide/ester carbonyl (δ_C 177.2 for C-1),

eight aromatic and olefinic carbons (δ_C132.5, 126.2, 136.6, 129.0×2, 129.3×2, and 127.5 for C-12, C-13, C-16, C-17/C-21, C-18/C-20, and C-19, respectively), one nitrogen bearing carbon (δ_C 64.2 for C-3), one sp^3 quaternary carbon (δ_C 59.0 for C-5), four methine carbons (δ_C 43.8, 30.2, 31.6, 38.0 for C-6, C-7, C-11 and C-14, respectively), four methylene carbons (δ_C 28.5, 19.6, 27.6 and 38.5 for C-8, C-9, C-10, and C-15, respectively), and two methyl carbons (δ_C 21.4 and 16.2 for CH_3-7 and CH_3-14, respectively). COSY correlations from H-6 to H-7 and H-11, from H-11 to H-10 and H-12, from H-7 to CH_3-7, and from H-14 to CH_3-14, helped set-up the octalin (octahydronaphthalene) ring system (Fig. 1A). Moreover, COSY correlations from the NH proton to H-3, and COSY correlations, as well as coupling constants, between H_2-15 and H-3 established the tetramic acid moiety. The key HMBC correlations (Fig. 1A) from the NH proton to C-5 and C-15, from H-6 to C-1, C-4, C-3, C-7, CH_3-7, C-10, and C-11, from H-13 to C-5, C-11, C-14, and CH_3-14, as well as from CH_3-14 to C-5, C-13, and C-14, suggested the spiro-ring juncture at C-5 between the octalin ring system and the 2,4-pyrrolidinedione ring system. The latter was a common structural motif in tetramic acids, of which many examples reside in the literature.[6] Although uncommon, the spiro-ring fusion between a polyketide-derived octalin ring system and a 2,4-pyrrolidinedione ring system has precedent in a few compounds, such as spylidone[7] and delaminomycins.[18] Compound **1**was ascribed the trivial name spiroscytalin.

Table 1. NMR data for compound **1** (500 MHz for ^1H, 125 MHz for^{13}C; δ in ppm; $CDCl_3$)

Position	**δ_H, m (*J* in Hz)**	**δ_C**	**DEPT**
1	—	177.2	C
3	3.78, dd (11.5, 3.0)	64.2	CH
4	—	212.2	C
5	—	59.0	C
6	2.28, br d (6.3)	43.8	CH
7	1.90, m	30.2	CH
8	1.38, br d (13.2)/1.27, m	28.5	CH_2
9	1.49, m/1.49, m	19.6	CH_2
10	1.78, m/1.55, m	27.6	CH_2
11	2.46, m	31.6	CH
12	5.77, dt (10.0, 3.0)	132.5	CH
13	5.34, dt (10.0, 2.0)	126.2	CH
14	2.73, m	38.0	CH
15	3.35, dd (14.0, 3.0)/2.51, dd (14.0, 11.5)	38.5	CH_2
16	—	136.6	C
17	7.22, d (7.5)	129.0	CH
18	7.35, t (7.5)	129.3	CH
19	7.29, d (7.5)	127.5	CH
20	7.35, t (7.5)	129.3	CH
21	7.22, d (7.5)	129.0	CH
7-CH_3	1.13, d (7.5)	21.4	CH_3
14-CH_3	0.89, d (7.5)	16.2	CH_3
NH	6.41, s	—	—

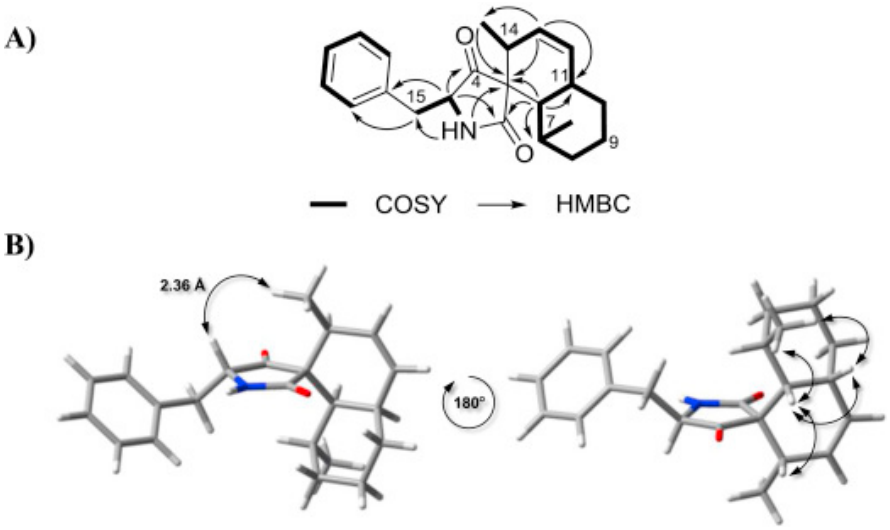


Fig. 1. A) COSY and selected HMBC correlations for compound **1**. **B**) Key NOESY interactions from the 'front' and 'back' view (180° turn through the *x*-axis) observed in the spectrum of **1** as noted using the DFT (B3LYP/DGDZVP) minimum energy model for **1**.

Solving the relative configuration of **1** was somewhat challenging, leading to two possibilities (structures **1** or **4**) that could fit the data. Due to overlapping signals in the ^{1}H NMR spectrum, many of the *J*-values were not informative. However, a coupling constant of 6.3 Hz between H-6 and H-11 suggested a *cis*-fused octalin ring system,[19] and this was confirmed by a strong NOESY correlation between these two protons (Fig. 1B). Strong NOESY correlations were also observed between H-6 and H-14, indicating a 1,3-pseudodiaxial relationship. A proton decoupling experiment was performed to confirm the *J*-value between H-6 and H-7. Irradiation of H-11 resulted in H-6 remaining as a broad doublet (*J*=5.2 Hz), indicating a *gauche* relationship between H-6 and H-7; this finding was supported by a strong NOESY correlation between H-6 and CH_3-7. The configuration at C-7 was enabled by a NOESY correlation between CH_3-7 and H-11, indicative of a 1,3-diaxial relationship. Finally, a NOESY correlation between H-3 and CH_3-14 suggested that they were on the same face of the molecule. At this stage, diastereomers **1** and **4** were both consistent with these NMR data, as were their respective enantiomers. Presumably, the configuration of the 2,4-pyrrolidinedione ring system depended upon whether an l- or d-phenylalanine was incorporated in the molecule.

Compound **1** exhibited an UV profile similar to spylidone,[7] and tetramic acids such as equisetin,[5] phomasetin,[5] and coniosetin[4] have been shown to display consistent circular dichroism spectra. Thus, electronic circular dichroism (ECD) calculations were carried out to assign the absolute configuration using density functional theory (DFT).[20, 21, 22, 23] Given the aforementioned relative configuration considerations, two distinct diastereoisomers were possible, either the 3*R*,5*S* (**1**) or 3*S*,5*R* (**4**). The respective enantiomers of these structures are also possible based on the data obtained, however, only structures **1** and **4** were utilized in the calculations since the ECD spectra would simply be antipodal for their enantiomers. Thus, a Monte Carlo conformational search, utilizing the MMFF94 molecular mechanics force field within a 5 kcal/mol energy window, led to three minimum conformers for each diastereoisomer. Reoptimization of the geometries of all conformers using DFT at the B3LYP/DGDZVP level, as implemented in the Gaussian 09 software, lead to the relative free energies and equilibrium

Boltzmann-weighted populations of the diastereoisomeric series incorporating either 3*R*,5*S*-**1** or 3*S*,5*R*-**4** (Fig. 2 and Tables S1 and S2). For further verification, calculations were also performed for the 3*S*,5*S* and 3*R*,5*R* diastereoisomers (data not shown); these two possibilities were ruled out because a NOESY correlation would not be possible from H-3 and CH_3-14. The three energy minimized conformers for the **1** and **4** series were submitted to ECD calculations by time-dependent density functional theory (TDDFT) at the B3LYP/DGDZVP level, considering the lowest 15 excited states with the CPCM solvent model for MeOH. The resulting ECD spectra for each series were energetically Boltzmann-weighted according to their respective conformational distribution and compared with the experimental ECD spectrum (Fig. 3 and Tables S1 and S2). The experimental ECD spectrum (Fig. 3) displayed a positive Cotton effect (CE) in the region of 210–230 nm and a negative CE in the region of 260–300 nm. These data were in good agreement with the CE calculated for the **1** series. Therefore, the independent TDDFT calculation method supported the absolute configuration of **1** as (3*R*,5*S*,6*R*,7*S*,11*R*,14*S*), suggesting the incorporation of l-phenylalanine, which was consistent with biosynthetic considerations.

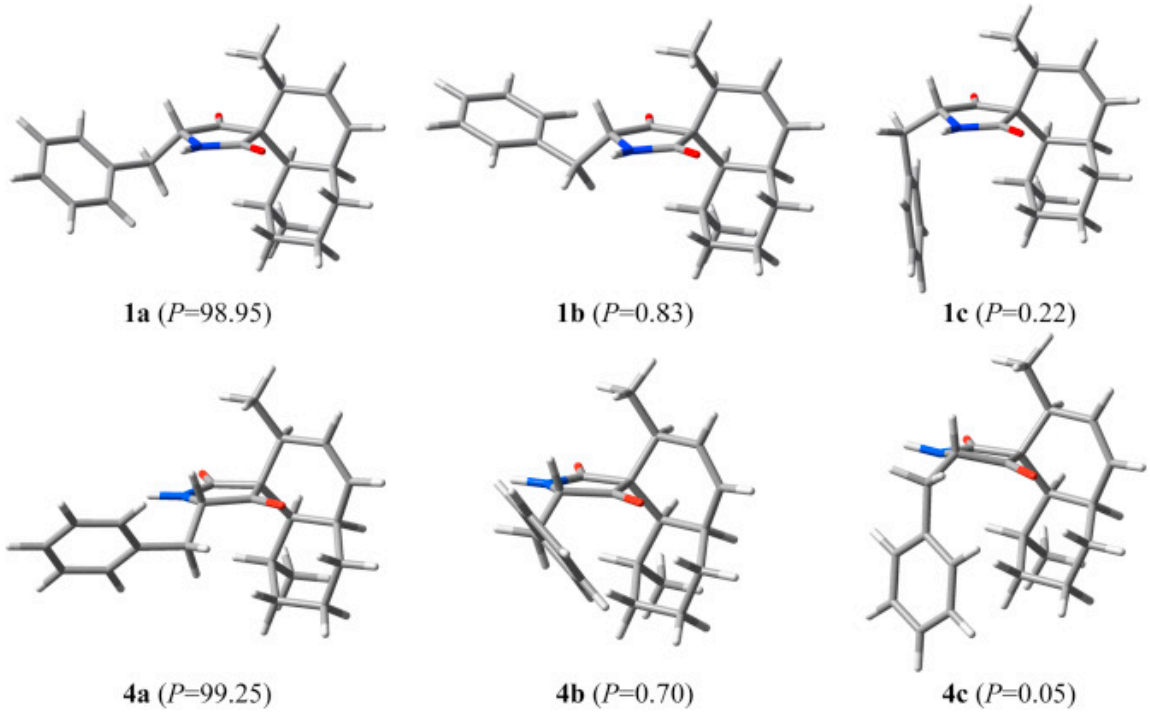


Fig. 2. DFT (B3LYP/DGDZVP) geometry optimized conformers of **1** and **4** showing equilibrium population percentages (*P*) based on Δ*G* (Gibbs free energies relative to the absolute *G* value for the global minimum), assuming Boltzmann statistics at *T*=298.15 K and 1 atm.

Compound **2** was assigned the molecular formula $C_{22}H_{25}NO_3$ on the basis of HRESIMS data (*m*/*z* 352.1909 for $[M+H]^+$), establishing an IHD of 11. The ^1H and ^{13}C NMR data (Fig. S2) of **2** matched that of a known compound leporin B,[17] which is a 2-hydroxy-4-pyridone alkaloid and also of mixed biogenetic origin.[6] Originally isolated from an unidentified fungal strain, **2** was reported as a transcription activator of hexokinase II gene, a possible target for the treatment of type 2 diabetes.[24] We attempted to determine the absolute configuration of **2** via crystallography by making a bromobenzoyl analogue. However, this was unsuccessful due to

paucity of material and the complex reactivity of the hydroxamate relative to a simple alcohol. Thus, relative configuration is shown for **2**.

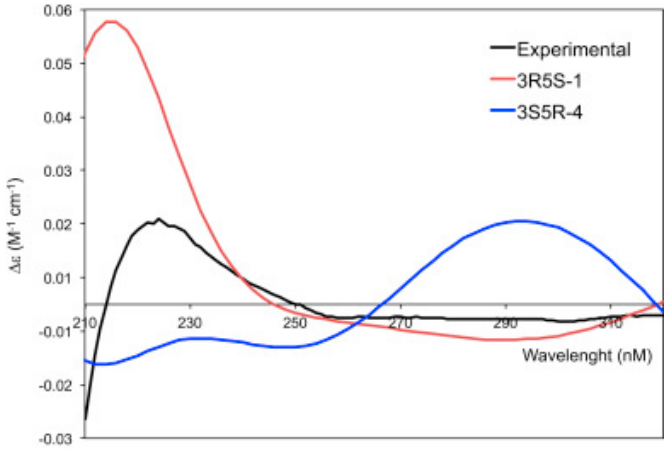


Fig. 3. Comparison of the experimental ECD spectrum of **1** with the calculated ECD spectra of (3*R*,5*S*)-**1** (**1a**–**c** series) and (3*S*,5*R*)-**4** (**4a**–**c** series).

Compound **3** was ascribed a molecular formula of $C_{23}H_{26}O_7$ based on HRESIMS data (*m*/*z*437.1563 for $[M+Na]^+$), indicating an IHD of 11. The ^1H (Fig. S3) and ^{13}C NMR and optical rotation data of **3** were in excellent agreement with those reported for purpactin A.[25] Purpactin A was first isolated from *Penicillium purpurogenum* and was reported to be an inhibitor of acyl-CoA:cholesterol acyltransferase.[25]

Compounds **1**–**3** were tested against three human tumour cells lines (Table 2). Compounds **1** and **3** were moderately active in the human tumour panel (i.e., IC_{50} values between 10 and 20 μM against all cell lines). The biological activity of the crude extract was likely due to leporin B (**2**), which displayed potent, sub μM activity against all three cell lines; cytotoxicity for **2** had not been reported previously. All three compounds were also tested for antimicrobial activity, and **2** showed strong activities against *Candida albicans* and *Staphylococcus aureus* (with MIC values of 6.25 and 25 μg/mL, respectively). Studies are ongoing to increase the supply of **2**, such that it can undergo more advanced testing.

Table 2. Cytotoxicity against a panel of human tumour cell lines of compounds **1**–**3**

Compound	**IC_{50} values (in μM)**		
	MCF7	**H460**	**SF268**
1	20.5	17.6	21.9
2	0.2	0.1	0.2
3	11.4	17.3	19.3
Camptothecin	0.07	0.01	0.03

3. Conclusion

A series of compounds of mixed biogenetic origin were isolated and characterized from a fungal culture, including a new tetramic acid analogue (**1**). The biological activities were most promising for the known, yet sparsely studied fungal metabolite, leporin B (**2**). The biosynthetic machinery utilized by the fungus to produce such compounds is intriguing, especially from the

standpoint of future studies to optimize the production of **2** and/or generate analogues via epigenetic manipulation of the growth conditions.

4. Experimental

4.1. General experimental procedures

UV spectra and optical rotations were acquired on a Varian Cary 100 Bio UV–vis spectrophotometer and a Rudolph Research Autopol III polarimeter, respectively. NMR spectra were recorded on JEOL ECA-500, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. HRESIMS was performed on a Thermo LTQ Orbitrap XL mass spectrometer equipped with an electrospray ionization source. HPLC was carried out using a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector, with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2). For preparative HPLC, a YMC ODS-A (5 μm; 250×20 mm) column was used with a 10 mL/min flow rate; for the semi-preparative HPLC, a YMC ODS-A (5 μm; 250×10 mm) column was used with a 5 mL/min flow rate; for analytical HPLC, a YMC ODS-A (5 μm; 150×4.6 mm) column was used with a 1 mL/min flow rate (all from Waters). For analytical HPLC, a MetaTherm HPLC column temperature controller (Varian) maintained the temperature at 30 °C.

4.2. Producing organism and fermentation

Mycosynthetix fungal strain MSX 68345 was isolated by Dr. Barry Katz of MYCOsearch in 1993 from leaf litter from a hardwood forest. The entire nuclear ribosomal ITS spacer region (ITS1-5.8S-ITS2)[26] and first 600 bp of the nuclear ribosomal Large Subunit (LSU) rRNA gene were sequenced using methods outlined previously.[27] Prior to BLAST search, the ITS region was edited using recent guidelines for establishing authenticity and reliability of ITS sequence data.[28] A BLAST search with the NCBI GenBank database using the ITS region revealed close identity with isolates of *S. cuboideum* (GenBank AB213451; Identities=599/613 (98%), Gaps=11/613; GenBank AB213444.1; Identities=595/612 (97%). Gaps=13/612). *S. cuboideum* represents a new name for a previously described fungus (*Arthrographis cuboidea*) based on recent phylogenetic analyses using SSU, ITS and RPB2 sequences.[29, 30] To confirm the identification of MSX 68345 using BLAST search, a Maximum Likelihood (ML) analysis was performed using RAxML.[31] The combined ITS and LSU sequence of MSX 68345 was subjected to a BLAST search and aligned with taxa with highest affinity from the GenBank BLAST search as well as with other searches implemented through the Fungal Barcoding project (http://www.fungalbarcoding.org/). The ML analysis indicated that MSX 68345 was phylogenetically related to a clade consisting of several isolates of *S. cuboideum*, including the type strain (CBS 241.62; AB213451, GQ272628) with strong ML bootstrap support (Fig. S4). Based on results obtained through BLAST search and phylogenetic analysis, MSX 68345 was identified as *S. cuboideum*(Helotiales, Leotiomycetes, Pezizomycotina, Ascomycota). The combined ITS-LSU sequence was deposited in GenBank (Accession No. KR920748).

The culture was stored on a malt extract slant and was transferred periodically. A fresh culture was grown on a similar slant, and a piece was transferred to a medium containing 2% soy peptone, 2% dextrose, and 1% yeast extract (YESD media). Following incubation (7 d) at 22 °C

with agitation, the culture was used to inoculate 50 mL of a rice medium, prepared using rice to which was added a vitamin solution and twice the volume of rice with H_2O, in a 250 mL Erlenmeyer flask. This was incubated at 22 °C until the culture showed good growth (approximately 14 d). The scale up culture was grown in a 2.8 L Fernbach flask containing 150 g rice and 300 mL H_2O and was inoculated using a seed culture grown in YESD medium. This was incubated at 22 °C for 14 d.

4.3. Extraction and isolation

To the large scale solid fermentation was added 500 mL of 1:1 MeOH/$CHCl_3$. The mixture was shaken for 24 h, filtered, and $CHCl_3$ and H_2O were added to generate a biphasic solution of 4:1:5 ($CHCl_3$:MeOH:H_2O). The solution was stirred for 2 h, transferred to a separatory funnel, and the organic (lower) layer was removed. This organic extract was evaporated and defatted by partitioning between hexanes and CH_3CN (1:1). The CH_3CN partition (494 mg) was fractionated via flash chromatography using silica gel via a hexanes/$CHCl_3$/MeOH gradient to afford six fractions. Fraction four (46.5 mg), which eluted with 0–5% MeOH in $CHCl_3$ over 18 min, was separated further by reversed-phase HPLC (20:80–100:0% CH_3CN–H_2O over 100 min) to obtain eight fractions. Fraction two yielded purpactin A (**3**; 8.0 mg), fraction three yielded the new compound spiroscytalin (**1**; 2.4 mg), and fraction four yielded leporin B (**2**; 6.5 mg). Two more scale-ups of the same extract were processed in the same way outlined above to obtain the same compounds with the following combined yields: Compounds **1** (7.5 mg), **2** (20 mg), and **3** (16 mg).

4.3.1. Spiroscytalin (**1**)

White powder; $[\alpha]_D^{25}$ −7.7 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε)=376 (6.28); ^1H, ^{13}C NMR, and HMBC data, see Table 1; HRESIMS *m*/*z* 338.2113 for $[M+H]^+$, calcd for $C_{22}H_{28}NO_2$, 338.2115.

4.3.2. Leporin B (**2**)

White powder; $[\alpha]_D^{25}$ −67.7 (*c* 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.60, s (H-3), 7.41, m (H-18/H-22), 7.37, m (H-19/H-21), 7.29, m (H-20), 5.73, dq (*J*=16, 7; H-15), 5.37, ddq (*J*=15, 8.6, 2; H-14), 4.82, dd (*J*=11, 9; H-13), 2.79, dd (*J*=10, 3.5; H-7), 1.77, m (H-8), 1.60, m (H-9a/H-9b), 1.51, m (H-10a), 1.39, tq (*J*=3.5, 13; H-10b), 1.76, m (H-11a), 1.60, m (H-11b), 1.72, m (H-12), 1.71, dd (*J*=7, 2; H_3-16), 0.86, d (*J*=6; H_3-23); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.2 (C-1), 129.2 (C-3), 111.2 (C-4), 157.6 (C-5), 113.7 (C-6), 38.1 (C-7), 35.9 (C-8), 35.8 (C-9), 20.9 (C-10), 26.5 (C-11), 35.2 (C-12), 78.2 (C-13), 129.8 (C-14), 131.2 (C-15), 17.9 (C-16), 133.7 (C-17), 129.1 (C-18/C-22), 128.3 (C-19/C-21), 127.5 (C-20), 20.4 (C-23); HRESIMS *m*/*z* 352.1909 for $[M+H]^+$, calcd for $C_{22}H_{26}NO_3$, 352.1907.

4.3.3. Purpactin A (**3**)

White powder; $[\alpha]_D^{25}$ −46.1 (*c* 0.01, $CHCl_3$); HRESIMS *m*/*z* 437.1563 for $[M+Na]^+$, calcd for $C_{23}H_{26}O_7Na$, 437.1571.

4.4. Computational methods

Theoretical calculations of TDDFT ECD spectra for compound **1** were performed with the Gaussian'03 program package.[32] Geometry optimizations for diastereoisomers **1** and **4**were carried out using the MMFF94 molecular mechanics force field calculations as implemented in the Spartan'08 program. A Monte Carlo search protocol[33] was carried out considering an energy cutoff of 5 kcal/mol, and this generated two sets of conformers, which were made up of three conformers each (**1a**–**1c** and **4a**–**4c**; Fig. 2). In each case, the minimum energy structures were filtered and checked for duplicity. No additional minimum energy structures were found. The conformers were optimized by DFT calculations at the B3LYP/DGDZVP level of theory, and thermochemical properties, IR, and vibrational analyses were performed at the same level. The self-consistent reaction field method (SCRF) with conductor-like continuum solvent model (COSMO) was employed to perform the ECD calculation of all conformers of **1**- and **4**-series in MeOH with the same basis set. The calculated excitation energy (in nm) and rotatory strength *R* in both dipole velocity (R_{vel}) and dipole length (R_{len}) forms were simulated into an ECD curve by using the following Gaussian function:

$$\Delta_\varepsilon(E) = \sum_{i=1}^{n}(E) = \sum_{i=1}^{n}\left(\frac{R_i E_i}{2.29 \times 10^{-39}\sqrt{\pi\sigma}} exp\left[-\left(\frac{E-E_i}{\sigma}\right)^2\right]\right)$$

where σ was the width of the band at $1/e$ height, and E_i and R_i were the excitation energies and rotatory strengths for transition i, respectively. σ=0.40 eV and R_{vel} were used.

4.5. Cytotoxicity assay

The cytotoxicity measurements against MCF-7[34] human breast carcinoma (Barbara A. Karmanos Cancer Center, Detroit, MI), NCI–H460[35] human large cell lung carcinoma (HTB-177, American Type Culture Collection, Manassas, VA), and SF-268[36] human astrocytoma (NCI Developmental Therapeutics Program, Frederick, MD) cell lines were performed as described previously.[37]

4.6. Antimicrobial assay

The compounds were screened for antimicrobial activity using an agar plate diffusion assay as described previously.[38]

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Supplementary data

Table S1. DFT (B3LYP/DGDZVP) atomic Cartesian coordinates (Å) for conformers **1**

Center number	1a				1b				1c		
	Coordinates (Angstroms)				Coordinates (Angstroms)				Coordinates (Angstroms)		
	X	Y	Z		X	Y	Z		X	Y	Z
1	6.567524	1.350420	0.219636		4.813565	2.620234	-1.352140		-4.444670	1.072015	-2.323266
2	5.835402	0.572019	0.019002		4.681172	1.669064	-0.843203		-4.127263	0.693908	-1.354379
3	3.949299	-1.429677	-0.478098		4.346913	-0.778317	0.476106		-3.319821	-0.300251	1.127900
4	4.612305	0.576096	0.696925		3.610817	1.491832	0.034305		-3.435037	-0.520008	-1.274038
5	6.119900	-0.435303	-0.912755		5.585774	0.621442	-1.067366		-4.418631	1.415348	-0.189795
6	5.173045	-1.437885	-1.157666		5.413639	-0.604092	-0.404322		-4.014753	0.911903	1.053433
7	3.651886	-0.425620	0.459751		3.423163	0.266175	0.709415		-3.014931	-1.031106	-0.033842
8	4.405147	1.358106	1.424921		2.911950	2.306489	0.206560		-3.229708	-1.078640	-2.184881
9	7.071011	-0.440656	-1.439267		6.422130	0.759159	-1.747794		-4.961907	2.355266	-0.249912
10	5.387853	-2.226682	-1.874447		6.117715	-1.415126	-0.571074		-4.242596	1.461067	1.963908
11	3.222234	-2.215587	-0.671757		4.232302	-1.723790	1.001376		-3.014406	-0.685901	2.098000
12	2.317429	-0.399511	1.172947		2.273570	0.076103	1.663202		-2.270653	-2.343480	0.053544
13	1.837478	-1.381459	1.137978		2.644160	-0.346594	2.607630		-2.463530	-2.822559	1.019073
14	2.449125	-0.129109	2.226361		1.785942	1.028017	1.886946		-2.637159	-3.032817	-0.717475
15	1.345778	0.645543	0.570178		1.218327	-0.909202	1.103929		-0.727475	-2.297053	-0.089779
16	1.826518	1.636203	0.608757		1.706853	-1.885034	0.940861		-0.379688	-3.339236	0.002631
17	0.000767	0.672233	1.300464		0.593242	-0.395413	-0.189007		-0.014226	-1.415453	0.937942
18	-0.163818	1.187420	2.463971		1.275989	-0.336800	-1.284105		0.077240	-1.710010	2.182237
19	0.932291	0.347757	-0.787235		0.049597	-1.068686	1.947140		-0.243826	-1.731139	-1.334563
20	1.591214	0.240491	-1.550879		0.102269	-1.479694	2.873399		-0.553269	-2.054058	-2.244851
21	-0.391285	0.443701	-1.038554		-1.146050	-1.053476	1.291796		0.830004	-0.918997	-1.234179
22	-0.917835	0.330454	-2.146971		-2.225699	-1.335250	1.813771		1.455586	-0.435873	-2.179563
23	-1.178949	0.797513	0.248646		-0.934916	-0.703372	-0.200973		1.247498	-0.727413	0.247179
24	-1.720220	2.266518	0.158565		-1.253270	-1.950123	-1.105505		2.569544	-1.523600	0.535492
25	-1.869173	2.566833	1.203686		-0.692056	-1.766724	-2.031495		2.540395	-1.713548	1.616292
26	-2.349027	-0.157289	0.677123		-1.709343	0.545409	-0.767035		1.395199	0.735465	0.791159
27	-2.655790	0.282334	1.633549		-1.312459	0.609882	-1.787644		1.593668	0.556205	1.854367
28	-0.731384	3.279150	-0.448612		-0.739663	-3.289599	-0.544625		2.664710	-2.902760	-0.145103
29	-1.153136	4.287109	-0.377382		-0.914920	-4.085316	-1.276523		3.576677	-3.407933	0.189534
30	-0.534487	3.079578	-1.506732		-1.255695	-3.572281	0.378511		2.716100	-2.822093	-1.235591
31	0.221781	3.287895	0.088121		0.336316	-3.260967	-0.347830		1.823057	-3.548986	0.118654
32	-3.070347	2.348319	-0.523247		-2.716609	-2.045487	-1.480230		3.817198	-0.708673	0.264691
33	-3.359454	3.336428	-0.882182		-3.050518	-3.019032	-1.841318		4.740968	-1.280221	0.170814
34	-3.622793	-0.084269	-0.222462		-3.245207	0.351605	-0.943477		2.649532	1.513148	0.280754
35	-4.450922	-0.378463	0.440736		-3.531802	1.049623	-1.745382		2.867241	2.242167	1.076564
36	-3.908961	1.318070	-0.682438		-3.588104	-1.031926	-1.424691		3.854597	0.624429	0.160749
37	-4.861031	1.478893	-1.189523		-4.622615	-1.200221	-1.726840		4.806406	1.116986	-0.041343
38	-1.932933	-1.625801	0.986743		-1.365432	1.901835	-0.083987		0.106372	1.606538	0.730081
39	-0.896944	-1.597656	1.346194		-0.329702	1.828825	0.270433		-0.747664	0.925282	0.806374
40	-3.663198	-1.075323	-1.409848		-4.111123	0.736380	0.279411		2.446458	2.344336	-1.007626
41	-2.962911	-0.745665	-2.181664		-3.978065	-0.011833	1.064991		2.370244	1.670054	-1.864668
42	-4.667531	-1.040568	-1.851877		-5.166666	0.705946	-0.021347		3.340815	2.961887	-1.163410
43	-3.319484	-2.507923	-0.983295		-3.755019	2.124267	0.827326		1.195748	3.228572	-0.935923
44	-3.285443	-3.159736	-1.864919		-4.337882	2.327877	1.734243		1.046850	3.739399	-1.895267
45	-4.109972	-2.906843	-0.334284		-4.034715	2.898220	0.100389		1.336121	4.017317	-0.185242
46	-1.968997	-2.542548	-0.254162		-2.254500	2.210537	1.140202		-0.040777	2.386078	-0.594504
47	-1.182159	-2.242273	-0.957361		-2.023932	1.506890	1.949658		-0.226568	1.684233	-1.416772
48	-1.728858	-3.567715	0.054978		-2.001045	3.208770	1.519514		-0.930872	3.024586	-0.531042
49	-2.776948	-2.198471	2.142951		-1.393107	3.052468	-1.109971		0.027221	2.544484	1.951367
50	-3.848946	-2.211445	1.918933		-2.375530	3.178735	-1.577605		0.871815	3.239677	2.007404
51	-2.638185	-1.606341	3.054701		-0.667643	2.874988	-1.912250		0.012488	1.968217	2.883620
52	-2.475469	-3.228626	2.365426		-1.133105	4.003496	-0.630378		-0.889729	3.144170	1.916307

Table S2. DFT (B3LYP/DGDZVP) atomic Cartesian coordinates (Å) for conformers of **4**

Center number	4a				4b				4c		
	Coordinates (Angstroms)				Coordinates (Angstroms)				Coordinates (Angstroms)		
	X	Y	Z		X	Y	Z		X	Y	Z
1	6.259734	1.340374	-1.191065		4.360166	2.689455	0.898675		-4.669305	-0.232220	2.000003
2	5.591659	0.596757	-0.762857		4.288069	1.681868	0.495821		-4.196373	-0.303127	1.023160
3	3.864959	-1.314086	0.316540		4.092093	-0.895584	-0.558569		-2.981891	-0.509720	-1.480751
4	4.248412	0.562361	-1.152331		3.180454	1.321309	-0.281615		-3.198054	-1.259166	0.801846
5	6.078007	-0.327533	0.171453		5.301890	0.750783	0.750887		-4.594426	0.552539	-0.011810
6	5.209489	-1.284625	0.709019		5.199668	-0.541634	0.219315		-3.983246	0.444975	-1.266769
7	3.363960	-0.392609	-0.618639		3.064070	0.028621	-0.818066		-2.572628	-1.374667	-0.451834
8	3.885013	1.280235	-1.885454		2.399996	2.054223	-0.474631		-2.912166	-1.928978	1.609905
9	7.122394	-0.304103	0.472817		6.163961	1.029460	1.352045		-5.375305	1.290260	0.156733
10	5.577322	-2.009915	1.431007		5.984857	-1.270639	0.405637		-4.287721	1.100244	-2.079622
11	3.200323	-2.066181	0.737149		4.030012	-1.898965	-0.976326		-2.518785	-0.589684	-2.462148
12	1.901291	-0.401481	-1.017039		1.864798	-0.369712	-1.652367		-1.529991	-2.440196	-0.713543
13	1.801952	-0.220781	-2.093154		1.336027	0.515438	-2.015615		-1.850789	-3.383878	-0.253435
14	-0.266108	0.612255	1.689204		-1.475915	-1.649502	-1.306847		1.125510	-1.228154	1.569561
15	-0.522140	0.591365	2.900394		-2.488116	-2.084997	-1.871560		1.595332	-1.146436	2.711134
16	-0.774872	1.480011	-1.739083		0.530071	-0.652656	1.470459		1.119450	-0.953956	-2.003562
17	-1.278526	0.781671	0.555786		-1.433987	-0.925451	0.038606		1.578829	-0.424235	0.348868
18	-2.001172	2.156857	0.622577		-1.919010	-1.843773	1.196741		3.056971	-0.731071	-0.035445
19	-2.682420	2.151606	1.487161		-3.002965	-1.997177	1.083100		3.709229	-0.321962	0.751228
20	-2.393947	-0.326665	0.586959		-2.347058	0.352413	0.052068		1.503157	1.126372	0.606787
21	-3.041975	-0.024292	1.418551		-3.364334	-0.052745	0.113988		2.376347	1.321411	1.240655
22	-1.082354	3.380047	0.779283		-1.270790	-3.237029	1.256759		3.417014	-2.219809	-0.181143
23	-1.680882	4.296476	0.816101		-1.689732	-3.808024	2.092248		4.484446	-2.324807	-0.402711
24	-0.383181	3.465150	-0.055947		-0.189689	-3.170839	1.400471		2.857152	-2.694064	-0.990406
25	-0.512370	3.321707	1.710857		-1.470807	-3.800077	0.340660		3.220191	-2.761852	0.748452
26	-2.850000	2.242875	-0.614602		-1.705366	-1.066739	2.465763		3.336724	0.038247	-1.298010
27	-3.054352	3.225897	-1.033892		-1.504100	-1.618739	3.381656		4.082250	-0.357896	-1.984825
28	-3.275845	-0.255528	-0.698849		-2.130350	1.175460	1.360995		1.745232	1.920013	-0.712703
29	-4.284856	-0.603188	-0.427989		-3.079133	1.683781	1.592111		2.204244	2.882933	-0.438376
30	-3.428195	1.142662	-1.199895		-1.816803	0.299542	2.530166		2.722813	1.225707	-1.603488
31	-4.023090	1.285995	-2.100122		-1.641894	0.790391	3.485973		2.955724	1.701528	-2.554622
32	-1.939928	-1.779101	0.925590		-2.334620	1.243355	-1.226291		0.284956	1.664995	1.414750
33	-1.068992	-1.713654	1.586495		-2.140519	0.594906	-2.087451		-0.032888	0.880615	2.109570
34	-2.829943	-1.165912	-1.880855		-1.063125	2.305512	1.284507		0.469769	2.287502	-1.521452
35	-1.922604	-0.756102	-2.333286		-0.064187	1.861354	1.260875		0.054269	1.383228	-1.974478
36	-3.613959	-1.141645	-2.647415		-1.129097	2.902413	2.202368		0.760243	2.957062	-2.340608
37	-2.567934	-2.602309	-1.420404		-1.260914	3.190701	0.050296		-0.585969	2.946550	-0.629414
38	-2.216173	-3.199997	-2.269933		-0.463214	3.942044	0.007400		-1.486036	3.155435	-1.220230
39	-3.502076	-3.065835	-1.078332		-2.205840	3.743373	0.129742		-0.220016	3.916064	-0.266468
40	-1.518616	-2.610456	-0.303534		-1.243032	2.333650	-1.220061		-0.933449	2.020947	0.541927
41	-0.582815	-2.210073	-0.710196		-0.258389	1.858913	-1.301801		-1.367588	1.100928	0.139072
42	-1.307363	-3.638270	0.017347		-1.363117	2.963892	-2.110220		-1.701903	2.480874	1.175754
43	-3.038814	-2.497884	1.737620		-3.733962	1.852632	-1.458239		0.722156	2.859574	2.289174
44	-3.990431	-2.556836	1.197726		-4.068246	2.469912	-0.617173		1.124662	3.688812	1.696804
45	-3.228422	-1.975529	2.681923		-4.481018	1.064731	-1.605268		1.497104	2.558221	3.002945
46	-2.733050	-3.522642	1.977868		-3.735684	2.486422	-2.352509		-0.127460	3.248006	2.862572
47	1.457375	-1.377652	-0.804460		2.208363	-0.925818	-2.534427		-1.471681	-2.633946	-1.789651
48	-0.403045	0.771939	-0.698152		0.059609	-0.652059	0.245446		0.674776	-0.962383	-0.770807
49	0.974468	0.494261	1.147413		-0.207937	-1.750573	-1.782438		0.112013	-2.057994	1.195560
50	1.794399	0.440570	1.743656		-0.019049	-2.258429	-2.641110		-0.204695	-2.775753	1.840358
51	1.071701	0.691919	-0.303861		0.864547	-1.279338	-0.897622		-0.074568	-2.195053	-0.254114
52	1.555009	1.660932	-0.507868		1.425961	-2.144163	-0.510691		0.474261	-3.090529	-0.594712

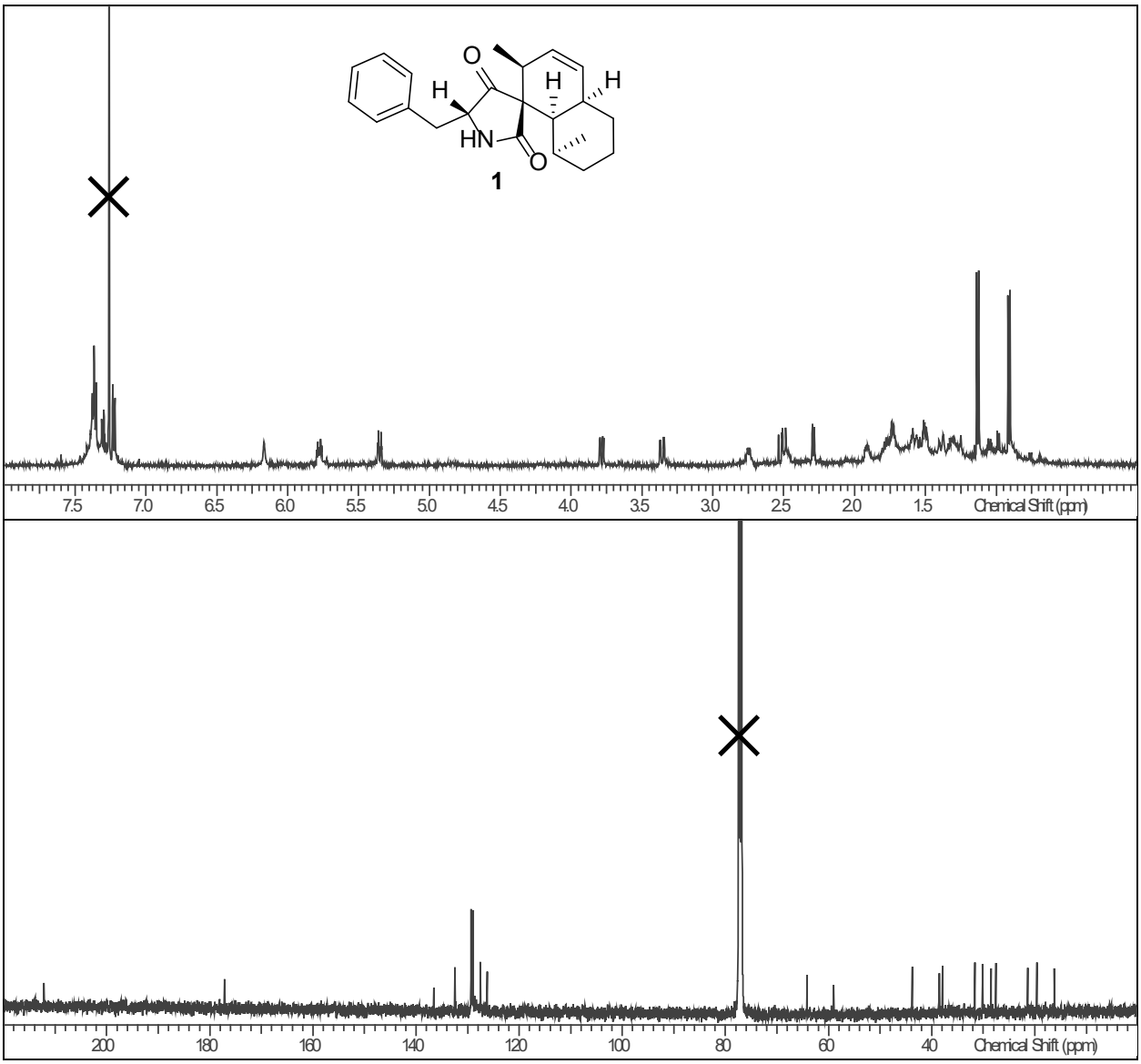

Fig. S1. ^{1}H (top) and ^{13}C (bottom) NMR spectra of compound **1** [500 MHz for ^{1}H and 125 MHz for ^{13}C; $CDCl_3$].

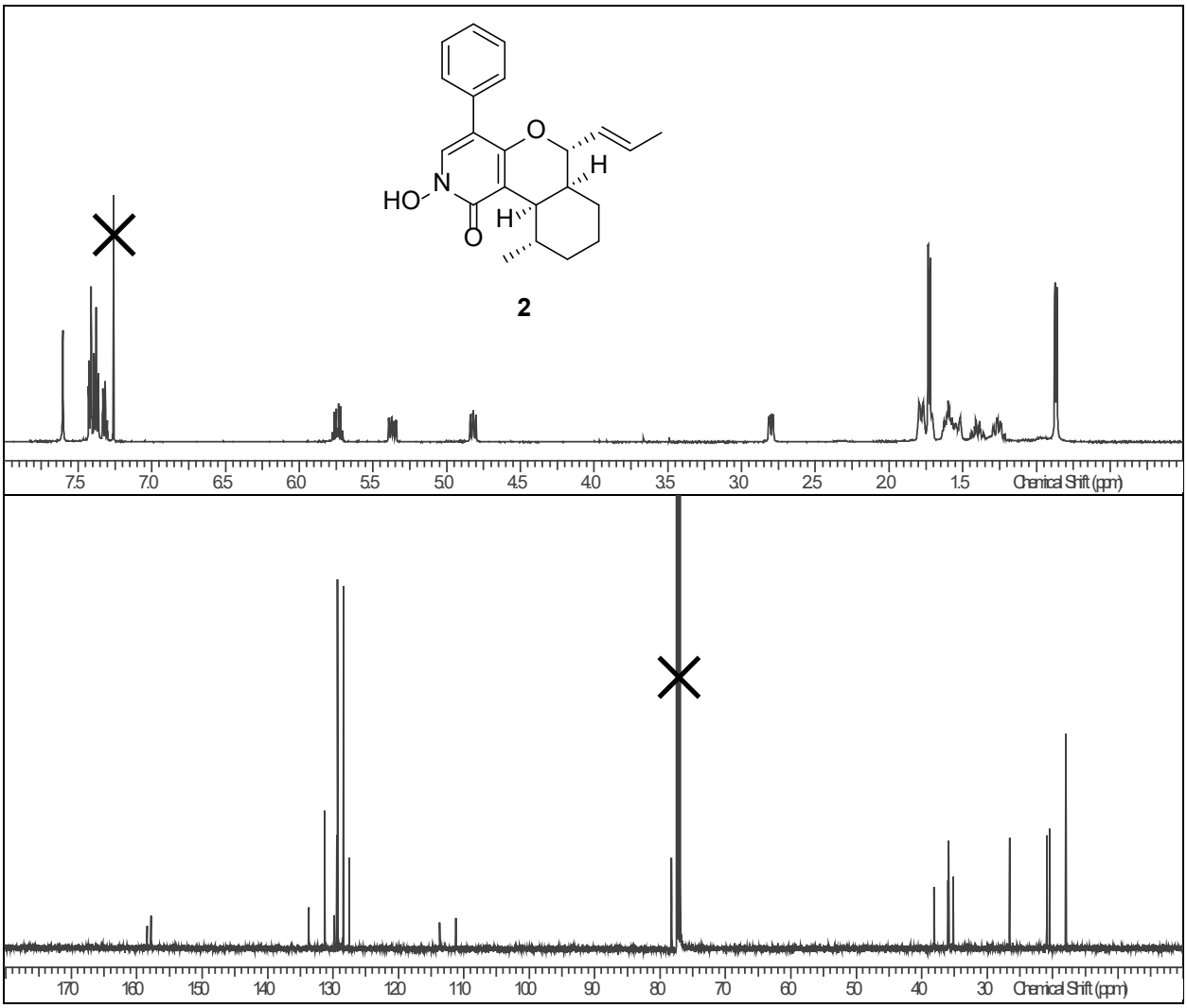

Fig. S2. ^{1}H (top) and ^{13}C NMR (bottom) spectra of compound **2** [500 MHz for ^{1}H and 125 MHz for ^{13}C; $CDCl_3$].

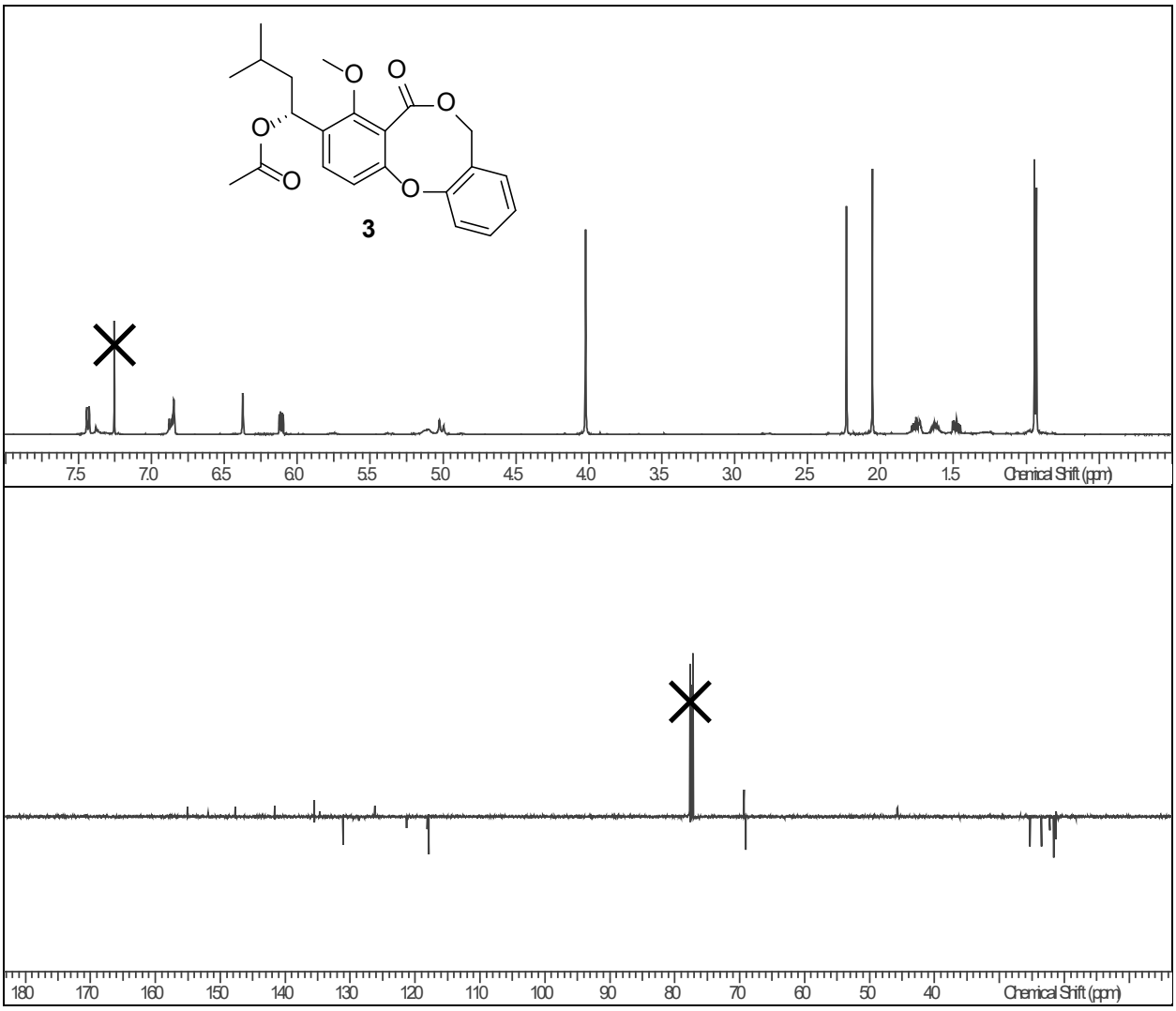

Fig. S3. ^{1}H (top) and DEPTQ (bottom) NMR spectra of compound **3** [500 MHz for ^{1}H and 100 MHz for ^{13}C; $CDCl_3$].

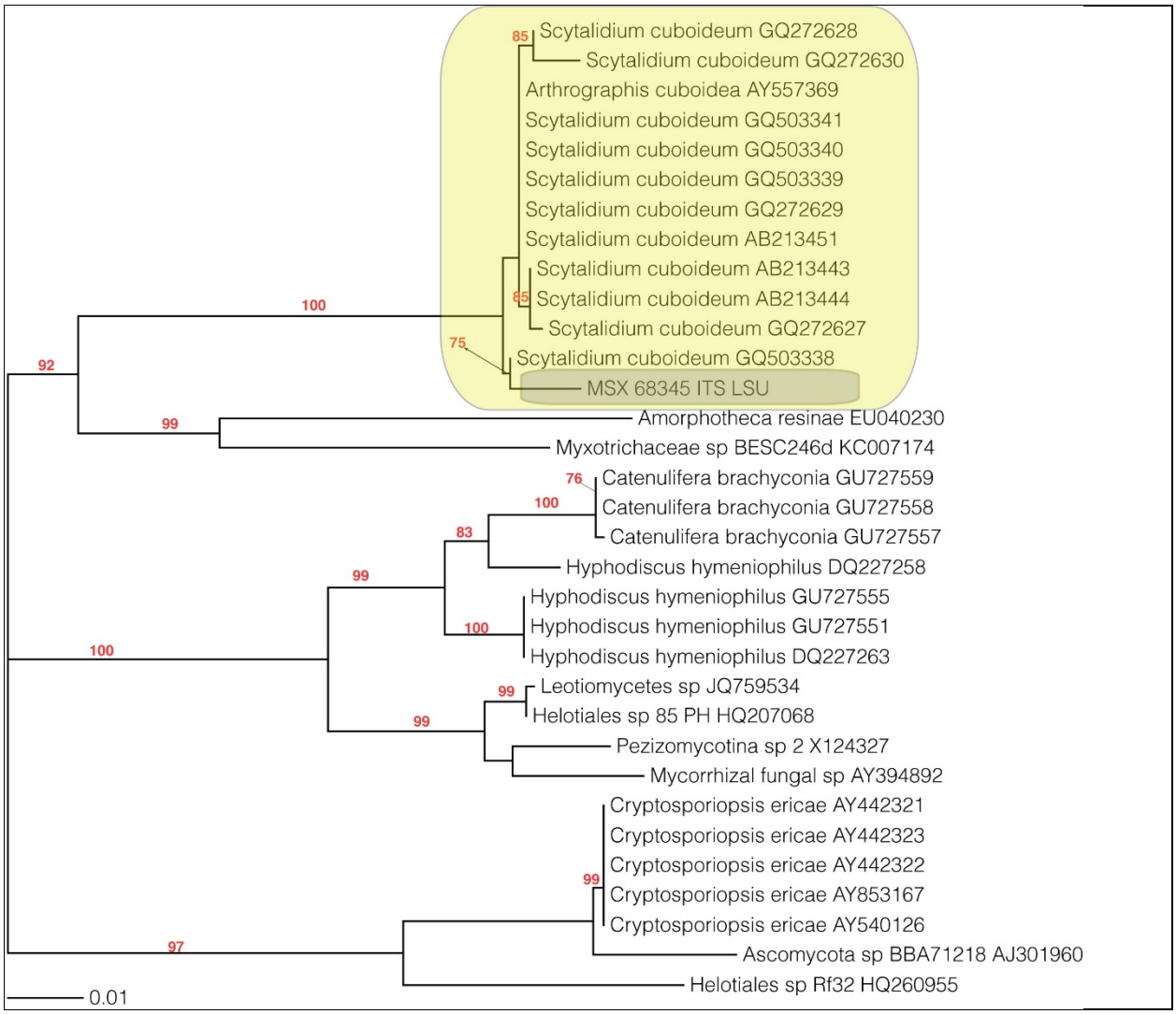

Fig. S4. Phylogram of the most likely tree (-lnL = 3472.03) from a RAxML analysis of 33 taxa based on complete ITS and partial region of the 28S large subunit nrDNA (1007 bp). Numbers refer to RAxML bootstrap support values ≥ 70% based on 1000 replicates. MSX 68345 is highlighted in gray and the *Scytalidium cuboideum* clade with 100% bootstrap value is highlighted yellow. Bar indicates nucleotide substitutions per site.